

## SEASONAL VARIATION IN SEX RATIO OF NESTLING ELEONORA'S FALCONS<sup>1</sup>

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**ABSTRACT.**—In a breeding colony of Eleonora's Falcon (*Falco eleonora*) on an island offshore of Crete, we determined the gender of 95% of the chicks by molecular (PCR) methods; 1028 samples were collected between 1997–2001. Hatching occurred between 11 August and 12 September with a variation of up to 5 d between years. The overall sex ratio was biased toward male fledglings (52.1%). The percent of males was positively related to the time of hatching. Falcons that hatched during the first 10 d of the hatching period had a higher daughter to son ratio. We propose that such bias may be adaptive because males with a higher fitness start to breed earlier and invest preferentially in female offspring than in lighter males. This hypothesis could explain some of the discrepancies in earlier sex-ratio studies on other raptors.

**KEY WORDS:** *Eleonora's Falcon; Falco eleonora; molecular sexing; sex allocation; sex ratio; male fitness.*

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### VARIACIÓN ESTACIONAL EN EL COCIENTE DE SEXOS EN PICHONES DE *FALCO ELEONORAE*

**RESUMEN.**—En una colonia reproductiva de halcones *Falco eleonora* en una isla cercana a la costa de Creta, determinamos el género del 95% de los pichones por medio de métodos moleculares (PCR) empleando 1028 muestras colectadas entre 1997–2001. La eclosión se produjo entre el 11 de agosto y el 12 de septiembre con una variación de hasta 5 días entre los años. El cociente de sexos global estuvo sesgado hacia volantones machos (52.1%). El porcentaje de los machos estuvo positivamente relacionado con el momento de la eclosión. Los halcones que eclosionaron durante los primeros 10 días del período de eclosión tuvieron un cociente mayor de hijas a hijos. Proponemos que este sesgo podría ser adaptativo ya que los machos con una adecuación mayor comienzan a reproducirse más temprano e invierten preferentemente en crías hembras que en machos más livianos. Esta hipótesis podría explicar algunas de las discrepancias observadas en estudios previos del cociente de sexos en otras rapaces.

[Traducción del equipo editorial]

The reason why primary and secondary sex ratios in raptors and other birds can deviate from parity has been widely debated (Bennet and Owens 2002, Hardy 2002, Komdeur and Pen 2002). Because it is often difficult to monitor a large number of nests within a single season, some studies suffer from small sample size. Several studies are biased toward species with a pronounced gender size dimorphism because this gender difference was formerly used to sex fledglings. As a consequence, de-

viations from parity tended to be discussed in relation to body size, the main explanations focusing on the advantage for female nestlings in food competition with male nestlings or on the advantage for males because of their smaller size and lower food requirements. Several obstacles which limited the selection of study species and timing of such studies have been overcome with the introduction of molecular techniques for gender determination (Ellegren and Sheldon 1997).

We selected Eleonora's Falcon (*Falco eleonora*) for a sex-ratio study because this species breeds in colonies with broods of 1–3 nestlings, so that a sufficiently large sample size could be obtained. In spite of its colonial habits, this falcon is monoga-

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mous (Swatschek et al. 1993). Furthermore, egg laying starts at the end of July during the stable weather conditions of the Mediterranean summer so that breeding performance rarely differs between years (Walter 1979, Wink et al. 1985, Wink and Ristow 2000). This falcon is insectivorous during the courtship period when it feeds upon unpredictable food patches far away from the breeding cliff, but is mainly an avian predator during the brood-rearing period in September, when it feeds upon autumn-migrant passerine captured over the sea. Typically, half of the falcon population is 7 yr and older, and males begin to breed at 3 yr of age (Ristow et al. 1989).

#### METHODS

**Field Work.** Eleonora's Falcon is a species of conservation concern and included in Annex 1 of the European Union's Wild Bird Directive. Thus, to avoid disturbances caused by measuring eggs or trapping adults, we restricted our sampling to the period when the young are older than 10 d. Under natural conditions, egg losses are fairly high and nestling mortality comparatively low.

We studied a colony of about 150 falcon pairs on an island off Crete (<1 km<sup>2</sup> in size) between 1997–2001. Nests were visited once as a rule in mid-September. In ca. 20% of situations young were too small for measurement at the first visit, and these nests were revisited a second time about two weeks later. Thus, more than 95% of the fledglings of the colony were banded annually, their wing chord measured, and blood samples of ca. 50 µl each were taken and stored in DNA buffer (EDTA buffer) in a vial.

For both genders, wing chord was converted into hatching date by means of the growth curve formula

$$A = \frac{WC + 52.1}{9.71} \quad \text{for } 45 < WC < 113 \quad \text{and}$$

$$A = \frac{WC - 8.3}{6.16} \quad \text{for } 113 < WC < 242$$

in which WC is the wing chord in mm and A is the age of a nestling in days. The accuracy of this formula is  $\pm 1$  d (Wink et al. 1991). In some nests with three young, the third bird may experience extended periods of lower growth rate than its older siblings. In such cases the application of the growth formula would give an age difference in excess of 5 d between second and third nestling, a value which would exceed the maximum difference between egg laying dates (Wink et al. 1985); differences between hatching dates should be smaller or equal to this value in our population (Wink et al. 1991). In such cases (ca. 2% of cases) simply a 5-d age difference was assumed for the runt young. A calculated age difference of up to 7 d was accepted in nests with two young if an unhatched egg explained the gap (ca. 1% of cases).

**DNA Isolation.** Blood samples were preserved in an EDTA buffer (0.1 M Tris, pH 7.4, 10% EDTA, 1% NaF, 0.1% thymol; Wink 1998) and stored at  $-20^{\circ}\text{C}$  until processing. Total DNA was extracted from the blood samples

by an overnight incubation at  $37^{\circ}\text{C}$  in lysis buffer (10 mM Tris [pH 7.5], 25 mM EDTA, 75 mM NaCl, 1% SDS) including 1 mg of Proteinase K (Merck, Darmstadt, Germany), followed by a standard phenol-chloroform protein extraction. DNA was precipitated from the supernatant with 0.8 volume of cold isopropanol, centrifuged, washed, dried, and resuspended in TE buffer (10 mM Tris-Cl, pH 7.5; 1 mM EDTA).

**Molecular Sexing.** Molecular sexing was modified (Becker and Wink 2002) according to the methods outlined in Kahn et al. (1998), which are based on the detection of the CHD gene on avian sex chromosomes. In most species, males produce one DNA band and females two, presumably reflecting differing intron sizes of the W versus Z chromosomes (Kahn et al. 1998). Polymerase chain reaction (PCR) used were 1237L: GAG AAA CTG TGC AAA ACA G and 1272H: TCC AGA ATA TCT TCT GCT CC. PCR conditions: the PCR mix consisted of 60 ng (2 µl) total DNA in 25 µl total volume, 0.12 µl 1272H Primer (97.45 pmol/µl), 0.103 µl 1237L Primer (83.1 pmol/µl), 1 µl nucleotide-mix (100 µM of GTP, CTP, TTP, and ATP), 2.5 µl 10× buffer with 15 mM MgCl, 0.15 µl Taq-Polymerase (0.6 Units; Pharmacia Biotech, Freiburg, Germany), and 0.1 µl <sup>33</sup>P α-dATP (1 µCi). PCR program: 2 min at  $94^{\circ}\text{C}$ , 31 cycles with 30 sec at  $94^{\circ}\text{C}$ , 1 min at  $56^{\circ}\text{C}$ , 2 min at  $72^{\circ}\text{C}$ , and finally 10 min at  $72^{\circ}\text{C}$ .

After 32 cycles the reaction temperature was maintained at  $72^{\circ}\text{C}$  for 4 min and then lowered to  $4^{\circ}\text{C}$  for further storage. PCR products were separated electrophoretically on a denaturing Sequagel matrix at 65 W for 1.5 hr (length 40 cm). After drying, the gel was exposed to an X-ray film (Hyperfilm-MP, Amersham, Freiburg, Germany), for 1–2 d, and developed (X-ray developer and fixer, Kodak, New Haven, CT U.S.A.).

#### RESULTS

The gender of 1028 young falcons from 556 nests was determined by molecular sexing (Table 1). As the number of infertile eggs and premature deaths amount to about 10% and <2%, respectively (Ristow and Wink 1985, Ristow et al. 1989, Wink et al. 1991), our data mostly reflect the primary sex ratio. There was a tendency to a higher percentage of sons as compared to daughters when all broods were considered (52.1% compared to the expected frequency of 50%; chi-square test,  $P < 0.1$ ).

The mean hatching date varied by 5 d between years (Table 2). If the data are corrected for year-to-year variation by setting the first hatching date as day 1, a positive correlation ( $r = 0.685$ ;  $N = 24$ ;  $P < 0.001$ ) was detected between the sex-ratio and the date of hatching. Daughters were more abundant during the first 10 d of the hatching period, whereas sons dominated in the middle and final period (Fig. 1).

Table 1. Seasonal variation of sex ratios in Eleonora's Falcons. Numbers of sons (M) and daughters (F) in relation to hatching date and year (Crete, Greece, 1997-2001).

DATE	1997		1998		1999		2000		2001		TOTAL			PERCENT	
	M	F	M	F	M	F	M	F	M	F	M	F	M + F	M	M
11 Aug	—	—	—	—	—	—	—	—	—	—	—	—	1	—	0.0
12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
13	3	2	—	—	—	—	—	—	—	—	—	—	6	—	66.7
14	4	3	1	1	—	—	—	—	—	—	—	—	10	—	50.0
15	1	3	4	1	—	—	—	—	—	—	—	—	11	—	45.5
16	4	4	2	6	—	—	—	—	—	—	—	—	27	—	33.3
17	4	8	3	4	3	2	1	2	2	4	4	20	33	—	39.4
18	6	5	2	4	1	—	—	3	—	3	3	15	30	—	50.0
19	8	11	4	9	—	2	3	6	4	6	4	32	53	—	39.6
20	6	10	5	12	—	5	6	6	11	5	22	44	66	—	33.3
21	7	7	7	7	5	1	5	6	7	16	40	28	68	—	58.8
22	13	8	9	8	4	2	6	4	12	12	44	32	76	—	57.9
23	13	4	6	4	6	4	7	6	4	4	36	22	58	—	62.1
24	13	6	8	9	3	6	8	4	5	5	37	34	71	—	52.1
25	3	9	10	6	7	10	11	8	8	8	39	39	78	—	50.0
26	4	6	10	9	8	11	10	3	7	7	39	34	73	—	53.4
27	5	7	11	3	5	5	4	5	3	3	28	26	54	—	51.9
28	4	3	5	7	10	7	7	9	5	5	31	27	58	—	53.4
29	10	2	5	8	6	7	3	4	5	5	29	22	51	—	56.9
30	3	2	7	6	5	5	10	5	2	2	27	19	46	—	58.7
31	1	1	2	3	10	3	4	4	3	3	20	12	32	—	62.5
1 Sept	1	1	8	2	6	4	2	2	—	—	18	9	27	—	66.7
2	4	1	2	2	6	7	4	4	2	2	18	15	33	—	54.5
3	3	2	—	1	3	3	2	1	2	2	10	8	18	—	55.6
4	1	2	—	2	3	1	—	1	2	1	5	8	13	—	38.5
5	—	1	2	3	1	2	—	—	—	4	7	7	14	—	50.0
6	—	—	3	—	—	—	—	—	—	—	3	—	3	—	100.0
7	1	—	—	—	1	1	—	1	—	1	3	2	5	—	60.0
8	1	1	—	1	—	—	1	—	—	—	2	2	4	—	50.0
9	1	—	1	—	—	—	—	—	—	—	2	1	3	—	66.7
10	—	1	—	—	—	—	3	—	—	—	3	1	4	—	75.0
11	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Sons	124	—	117	—	93	—	102	—	100	—	536	—	—	—	100.0
Daughters	110	—	118	—	86	—	86	—	87	—	492	—	—	—	—
Total	234	—	235	—	184	—	188	—	187	—	1028	—	—	—	—

Table 2. Yearly variation of hatching dates of sons and daughters, and the percentage of males in Eleonora's Falcon broods (Crete, Greece, 1997–2001). The median hatch dates are used to characterize the center of the asymmetrical yearly distributions.

YEAR	MEDIAN FOR SONS	MEDIAN FOR DAUGHTERS	DIFFERENCE	PERCENTAGE OF MALES
1997	23.5	22.3	1.2	53.0
1998	25.7	24.3	1.4	49.8
1999	28.5	27.0	1.5	50.5
2000	26.0	25.6	0.4	54.3
2001	22.8	21.7	1.1	53.5
Total	24.4	23.5	0.9	52.1

#### DISCUSSION

This study was carried out during a period of population decline caused by poisoning of falcons on mainland Crete resulting with instant death of many adults (Ristow 2001, Anonymous 2002); thus, the number of nestlings sampled decreased from 234–187 between 1997–2001 (Table 1). We assume that this effect was not responsible for the observed gender bias, which was present within each year (Table 2). Nor did we find evidence of a gen-

der bias in embryo mortality, although our data from unhatched eggs are few (3 males, 3 females).

We interpret the early bias toward daughters in the first third of the hatching period to reflect to some degree the fitness of parents and of males in particular. From previous studies on fitness in Eleonora's Falcon, Wink et al. (1985) established that large and heavy males have larger clutches and that mass between partners was uncorrelated. Mass of males (and likely fitness) increases with age, three-egg clutches tend to be started earlier in the breeding period than smaller clutches, and the first egg laid is the heaviest within a clutch (Wink et al. 1982a, 1985, 1991). The sum of these details was that experienced and successful pairs tended to start clutches early, and these produced more daughters. Vice versa, light males apparently produce more sons. In the case of Eleonora's Falcon, our results may be interpreted that the heaviest (fittest) males can afford to invest into the rarer gender, which needs a larger food supply (daughters were 15–20% heavier than sons; Wink et al. 1982b, 1991). This interpretation is in line with the observation that 11 pairs had three daughters each as compared to only seven pairs with three sons each.

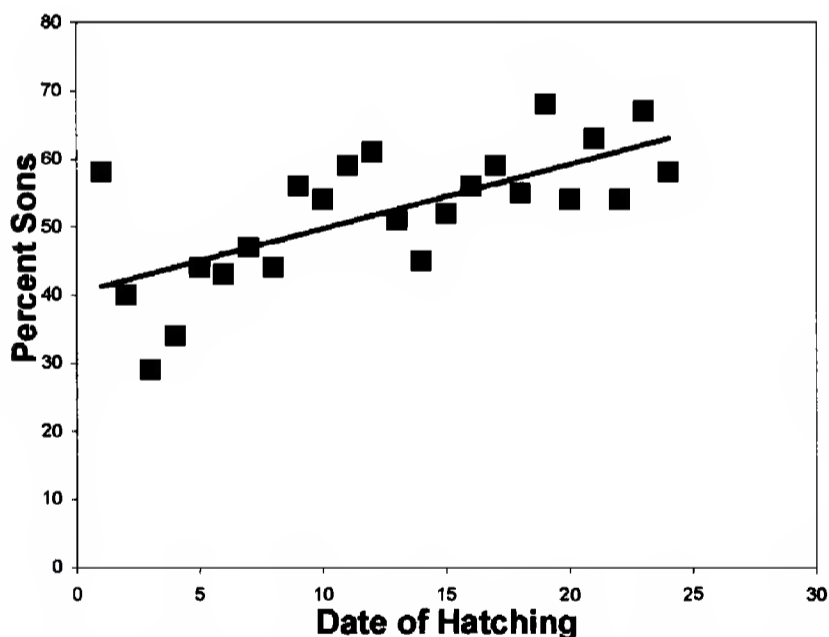


Figure 1. Seasonal variation of sex ratio in Eleonora's Falcons ( $N = 1028$  fledglings, Crete, Greece, 1997–2001). The Y-axis indicates mean daily percentage of males and the X-axis standardized days of hatching. The following standardization procedure was adopted: 13 August was set as day 1 for 1997 as the first year; the distributions of the following years in Table 1 were shifted by 2, 5, 3, and 0 d, respectively toward earlier dates (Table 2). Then, all broods earlier than day 1 were pooled with those of day 1. Similarly, 5 September was set as day 24 in 1997 and all later broods were pooled with those of day 24. Regression equation:  $Y = 0.94x + 40.33$ ;  $r^2 = 0.469$ ,  $P < 0.001$ .

If the above interpretation is valid, then the age of the adult males should be taken into account when comparing sex ratios among species. For example, no skewed sex allocation was found in Peregrine Falcons (*Falco peregrinus*; Burnham et al. 2003) in North America. The reevaluation of a study with German peregrines (data from Fig. 62 and Table 31, Rockenbach 2002) also did not reveal a statistically significant trend in sex ratios. These data sets were obtained in populations that were recovering after severe declines in the 1960s and 1970s and which consisted of a large percent-

age of young pairs. This might explain why in Australia, where such a decline had not taken place, a female-biased sex ratio was reported (Olsen and Cockburn 1991).

In Eurasian Kestrels (*Falco tinnunculus*) the proportion of sons increased with later laying in years of low and moderate food supply, whereas in years of good food supply the sex ratio was son biased throughout (Korpimäki et al. 2000). In this Finnish study of typically 30 falcon pairs, the population turn-over was >75% per yr, so that young breeders dominated. Also, in years of good food supply kestrels of inferior fitness could reproduce without chick loss. We suggest that this pattern is similar to what we observed for Eleonora's Falcons, in that less-fit males seemed to breed later in the season and produced male-biased broods.

In the American Kestrel (*Falco sparverius*), no trend or the opposite seasonal trend in the sex ratio of fledglings was reported (Anderson et al. 1997, Smallwood and Smallwood, 1998, Griggio et al. 2002). A simple explanation for this deviation from the other falcon species was not evident to us, but the extended laying season of 4 mo and the fact that males of this small falcon breed at 1 yr of age may be of relevance. Also in Lesser Kestrels (*Falco naumanni*) a secondary sex-ratio bias toward daughters as the breeding season progressed has been reported (Tella et al. 1996). Information about parents' age distribution would have helped to integrate these differing results into our suggested broader concept for falcons.

After having discussed the available data on falcon species that agree with or do not agree with our interpretation, it is worthwhile to examine data for other raptor species. Age of parents had been considered in the sex allocation of the Eurasian Sparrowhawk (*Accipiter nisus*; Risch and Brinkhof 2002), and these results were consistent with our interpretation of the Eleonora's Falcon data. However, neither parents' age nor fitness can explain the mechanism of how birds skew their offsprings' sex ratio. This becomes obvious when non-raptors are considered. For example, Cory's Shearwater (*Calonectris diomedea*) nesting on the same study island showed the opposite seasonal trend in sex ratio as compared to the Eleonora's Falcon (D. Ristow and M. Wink unpubl. data).

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